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Original Article

Feasibility of anti-HCV reflex HCV Ag screening strategy in an HCV endemic community

Wei-Ming Chen [a,b,c,1], Chih-Yi Lee [d,1], Nien-Tzu Hsu [e], Wei-Cheng Huang [f], Mei-Yen Chen [g], Tung-Jung Huang [h], Sheng-Nan Lu [a,e,*]

[a] *Division of Gastroenterology and Hepatology, Department of Internal Medicine, Chang Gung Memorial Hospital, Chiayi, Taiwan*
[b] *Department of Health and Nutrition, Chia Nan University of Pharmacy and Science, Tainan, Taiwan*
[c] *School of Medicine, College of Medicine, Chang Gung University, Taoyuan, Taiwan*
[d] *Department of Laboratory Medicine, Chang Gung Memorial Hospital, Chiayi, Taiwan*
[e] *Biostatistics and Bioinformatics Center of Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan*
[f] *Department of Geriatrics, Chang Gung Memorial Hospital, Chiayi, Taiwan*
[g] *Department of Nursing, Chang Gung University of Science and Technology, Chiayi, Taiwan*
[h] *Department of Pulmonary Disease and Critical Care, Chang Gung Memorial Hospital, Yunlin, Taiwan*

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Background/Purpose: The HCV core antigen (HCV Ag) assay displays high sensitivity and strong correlation with HCV RNA. However, the feasibility of anti-HCV reflex HCV Ag screening in a community-wide setting is rarely discussed.
Methods: We performed a two-phase community-based hepatitis C screen in an HCV-prone area of central Taiwan. During the training phase, all participants were test for anti-HCV, HCV Ag and HCV RNA to validate sensitivity, specificity, and accuracy of HCV Ag. During the validation phase, an anti-HCV reflex HCV Ag screen was conducted based on the results of training phase. Outcomes of the study were presented as positive and negative predictive values (PPV and NPV).
Results: Of 935 training phase participants, the rate of positive anti-HCV and HCV Ag were 175 (18.7%) and 78 (8.3%), respectively. Test sensitivity, specificity, and accuracy of HCV Ag were 97.1%, 98.6%, and 97.8%, respectively. During validation phase, only anti-HCV-positive serum samples were tested for HCV Ag. Of 1932 participant, 285 (14.8%) were anti-HCV-positive. 133 (46.7%) of the 285 anti-HCV-positive samples were HCV Ag-positive. PPV and NPV were

* Corresponding author. Division of Hepatogastroenterology, Department of Internal Medicine, Chiayi Chang Gung Memorial Hospital, No.6, Sec. W., Jiapu Rd., Puzi City, Chiayi County, Taiwan. Fax: +886 5 3622188.
E-mail address: juten@ms17.hinet.net (S.-N. Lu).
[1] These authors contributed equally.

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98.4% and 99.3%, respectively. Across the entire participant sample, a significant linear correlation between HCV Ag and HCV RNA concentration was noted ($r^2 = 0.93$, p-value$<$0.001) following log–log transformation.
Conclusion: Anti-HCV reflex HCV Ag screening is a feasible strategy for aiding HCV-prone communities.

Introduction

Hepatitis C virus (HCV) infection is a global public health issue. Chronic hepatitis C infection is a leading cause of liver-related morbidities such as liver cirrhosis, liver failure, and hepatocellular carcinoma (HCC).[1] HCV is also associated with extra-hepatic conditions such as autoimmune disease and metabolic syndrome.[2,3] Interferon-based therapy and direct-acting antivirals (DAAs) are effective treatments for HCV patients,[4,5] and many DAAs regimens display high rates of safety, tolerability and sustained virologic response (SVR) (>97%). Moreover, DAAs regimens can improve liver stiffness, reduce the incidence of HCC, and improve survival.[6–8] However, most active infected hepatitis C patients are asymptomatic and unaware of their infection. Such ignorance is a major source of disease transmission.[9] Hepatitis C has been noted to aggregate within certain communities,[10–12] making community-based screening of infected HCV patients key in achieving the global goal of eradicating HCV infection as a major public health threat by 2030.[13,14]

The conventional way to diagnose an active HCV infection is through the two-step method. First, the anti-HCV antibody is tested for by serological immunoassays to evaluate the presence of an active infection. Second, a more expensive "gold standard" HCV RNA molecular diagnosis is performed, which employs polymerase chain reaction (PCR) technology to confirm active HCV infection.[15,16] This two-step method is often too expensive and time-consuming to be used for community-wide screening.

Hepatitis C virus core antigen (HCV Ag) is a viral protein released into the plasma during viral assembly, and is encoded by the 5' RNA terminal sequence of the HCV genome.[17,18] Moreover, HCV Ag is highly conserved and is associated with the presence of complete viral capsules for all known viral genotypes.[18,19] As with HCV RNA, HCV Ag is usually detectable in human serum or plasma 2–3 weeks following infection, and can be detected long before anti-HCV antibodies.[18,20] Many HCV Ag detection assays provide good correlation with HCV RNA in both hospitals and community screening, and are often used as an alternative to HCV RNA for the diagnosis of active HCV infection as well as to evaluate patient response to antiviral therapy.[15,17,21,22]

In 2019, the Taiwan Hepatitis C Policy Guideline 2018–2025 was published, officially recognizing Taiwan's commit to eliminating hepatitis C by 2025.[23,24] To reach this goal, community-wide screening using an accurate, effective, and cheap method was needed — the anti-HCV reflex HCV Ag screening strategy.

This study therefore aims to investigate the feasibility of the anti-HCV reflex HCV Ag screening strategy in HCV-prone communities.

Materials and methods

In 2018, a two-phase, community-wide hepatitis C screening was conducted in an HCV prone area of central Taiwan. During training phase, all participants were tested for anti-HCV and HCV Ag. Those positive for either anti-HCV or HCV Ag then underwent confirmation testing via HCV RNA. The aims of the first phase was to realize (1) the number of HCV Ag-positive individuals among anti-HCV-negative cases, and (2) to validate HCV Ag prediction of HCV viremia. Validity measures included sensitivity, specificity, and accuracy. During validation phase, an anti-HCV reflex HCV Ag screening strategy was conducted based on the results of training phase. The validation phase results are presented as positive and negative predictive values (PPV and NPV) of HCV Ag.

Laboratory tests were conducted as follows:

We used the Cobas e411 analyzer with an Elecsys Anti-HCV II assay kit (Roche Diagnostics GmbH, Mannheim, Germany) and an automated electrochemiluminescence immunoassay to detect anti-HCV antibody.
The HCV Ag detection assay was performed by using a two-step chemiluminescent microparticle immunoassay. Using the ARCHITECT HCV core antigen detection assay (Abbott Laboratories, Sligo, Ireland), we divided samples into a liquid phase with acridinium-labelled murine anti-HCV antibodies, and a solid phase with paramagnetic microparticles for quantitative measurement of the HCV antigen. Samples with concentration values $>$ 3.00 fmol/L were considered to represent reactivity for HCV Ag.
The HCV RNA was detected using the Cobas AmpliPrep/Cobas TaqMan HCV quantitative test v2.0 (Roche Molecular Systems, South Branchburg, USA). This assay demonstrated a lower limit of quantification of 15 IU/ml across all HCV genotypes.

Results

There were 935 participants included in training phase. The prevalence rates of anti-HCV and HCV Ag were 175 (18.7%) and 78 (8.3%), respectively. All 78 HCV Ag-positive participants were anti-HCV-positive and showed a positive rate of

44.6% (78/175). To elucidate the validity of HCV Ag in predicting the presence of HCV RNA, 139 anti-HCV-positive participants underwent another serological test to detect HCV RNA. The sensitivity, specificity, and accuracy of the test were 97.1%, 98.6%, and 97.8%, respectively (Table 1).

In training phase, anti-HCV reflex HCV Ag screening was conducted. During this phase, only anti-HCV-positive serum samples were tested for HCV Ag. Of 1932 participants, 285 (14.8%) were anti-HCV-positive. Of those, 133 (46.7%) were HCV Ag-positive. Among the 285 anti-HCV subjects, 273 residual samples were qualified enough to undergo HCV RNA testing. PPV and NPV were subsequently found to be 98.4% and 99.3%, respectively (Table 2).

412 participants provided results for both HCV Ag and HCV RNA testing, revealing a significant linear correlation between HCV Ag and HCV RNA concentrations ($r^2 = 0.93$, p-value$<$0.001) following log–log transformation (Fig. 1).

Discussion

Hepatitis C virus (HCV) infects about 2.5% of the human population and is a cause of hepatic morbidity and mortality.[25] In Taiwan, individuals positive for the anti-HCV antibody exist at a rate of 1.5–4.5%.[23,26] This number equates to around 500,000 people, most of whom are elderly and live in the south of Taiwan.[27,28] In our focal community of central Taiwan, the positive anti-HCV antibody rate was 16.04%. As previously reported, a positive HCV RNA rate among anti-HCV antibody-positive adult patients is expected to be 66–88% in Taiwan,[23] yet the HCV RNA positive rate observed in our study was 48% – a large drop from 10 years prior. Our finding may suggest the tendency to over-estimate the number of active cases.

Hepatitis C virus core antigen (HCV Ag) is a structural protein which exists in both complete HCV virions and RNA-free core protein structures.[29] There are five commercial HCV Ag assays available that detect HCV Ag nucleocapsid peptides 22 (p22) in plasma during viral assembly: 1) the Abbott ARCHITECT HCV Ag assay, an automated chemiluminescent microparticle immunoassay (CMIA), 2) the Fujirebio Lumipulse Ortho HCV Ag assay, 3) the EIKEN Lumispot HCV Ag assay, which resembles automated chemiluminescent enzyme immunoassays (CLEIA) available in Japan and China, 4) the Hunan Jynda Bioengineering Group HCVcAg ELISA, and 5) the Ortho ELISA-Ag. It should be noted that enzyme-linked immunosorbent assays (ELISA) are known for providing high sensitivity, high specificity, and strong correlation with HCV RNA above 3000 IU/mL.[15] In our study, we used the ARCHITECT HCV core antigen detection assay, finding the assay to provide high sensitivity (98.5%), specificity (98.6%), and significantly correlated with HCV RNA above 15 IU/ml among our Taiwanese participant pool. We also noted that HCV Ag concentrations significantly correlated with HCV RNA concentrations (Fig. 1). Interestingly, all positive HCV Ag cases were also positive for the anti-HCV antibody in our study, suggesting a single-step HCV Ag test may be able to replace both anti-HCV antibody and HCV RNA testing. With community-wide screens, it is cheaper to test for either anti-HCV antibody or HCV Ag. This economic incentive makes such tests ideal for evaluating anti-viral treatment and government policy efficacy alike. The positive predictive value (PPV) and negative predictive value (NPV) of HCV Ag in this study were as high as 98.4% and 98.6%, respectively, further suggesting that our testing method is feasible for community-wide screening.

HCV RNA reflex testing is the recommended method of following up a positive anti-HCV antibody test to confirm diagnosis of HCV infection.[30,31] Reflex testing refers to when a laboratory performs an HCV RNA test immediately on the same serological specimen upon positive anti-HCV antibody finding. If the subsequent HCV RNA test is negative, HCV infection can be ruled out. If the reflex HCV RNA test is positive, a diagnosis of HCV viremia is usually made, and the patient will often be referred for further medical

Table 1 Results between HCV core antigen and HCV RNA.

	HCV RNA (+)	HCV RNA (−)
In the training phase (n = 139)		
HCV Ag (+)	66	1
HCV Ag (−)	2	70
In the validation phase (n = 133)		
HCV Ag (+)	126	2
HCV Ag (−)	1	144

HCV Ag, hepatitis C virus core antigen.

Table 2 Validity of anti-HCV reflex HCV Ag screening.

	Training phase N = 139	Validation phase N = 133	Total N = 412
Sensitivity	97.1%		98.5%
Specificity	98.6%		98.6%
Accuracy	97.8%		98.5%
Positive Predictive Value		98.4%	98.4%
Negative Predictive Value		99.3%	98.6%

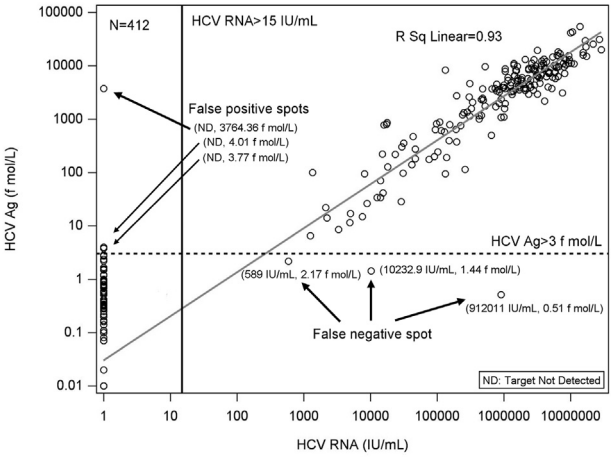

Figure 1 Linear correlation between HCV Ag and HCV RNA.

care. In this study, we performed HCV Ag testing in a similar reflex manner upon positive anti-HCV antibody finding, yielding high sensitivity, specificity, and PPV. A previous study found that anti-HCV test in conjunction with HCV Ag is cheaper than anti-HCV with HCV RNA testing for the purpose of identifying HCV viremia.[32] The anti-HCV reflex HCV Ag screening strategy is therefore a more efficient and economical testing option for community-wide HCV screening. As previously mentioned, viremia among anti-HCV -positive cases occurs at a rate of less than 50%. Reflex testing avoids the costs of medical referral, and HCV Ag test provides high PPV and NPV at a lower cost.

Pan-genotype direct-acting antivirals (DAAs) such as Maviret (glecaprevir/pibrentasvir), Epclusa (sofosbuvir/velpatasvir) and Vosevi (sofosbuvir/velpatasvir/voxilaprevir) are well tolerated and provide sustained virologic response (SVR) in whole genotype HCV infected patients. To eliminate hepatitis C infections globally, pan-genotype DAAs facilitate patient treatment 8—12 weeks after screening. Our previous clinical study found a strong correlation between HCV Ag and HCV RNA in both genotype 1 and genotype 2 patients.[22] Moreover, HCV Ag was found to be feasible in monitoring the therapeutic efficacy of DAAs among genotype 1 patients.[33] With our study, complete HCV genotyping might be not necessary given that we treated HCV Ag-positive individuals with pan-genotype DAAs.

The present study was not without limitations. First, the impact of HCV genotype on HCV Ag test results was not considered. From recent study, an increased odd of false-negative result was associated with genotype 3, and reduced odds of false negative were associated with older age and viral load.[34] Second, we did not consider the interaction between HCV Ag and concomitant infection such as hepatitis B, HIV, or mixed genotype HCV patients. Third, we did not perform further tests to exclude HCV genetic mutation or laboratory error in our false positive or false negative cases. Previous studies indicated genetic mutation and sample denaturation did lower the sensitivity of HCV Ag test.[16,35]

In conclusion, HCV Ag provided results similar to HCV RNA testing during a community-wide screen in central Taiwan. All individuals positive for HCV-Ag were also positive for the anti-HCV antibody. We therefore argue that testing for HCV Ag is sufficient as a follow-up diagnostic test for positive anti-HCV antibody findings. Moreover, the relatively lower cost of anti-HCV reflex HCV Ag testing makes it an ideal strategy for community-wide screening.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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